REMARKS

Following entry of the foregoing amendments, claims 29 to 51 will be pending in the application. Claim 29 has been amended, herein. No new claims have been added and no claims have been canceled.

Applicants respectfully request reconsideration of the rejections of record in view of the foregoing amendments and the following remarks.

Alleged Lack of Enablement

Claims 29 to 51 have been rejected under 35 U.S.C. § 112, first paragraph for lack of enablement because undue experimentation would allegedly be required to practice the methods defined by the claims. Applicants respectfully traverse the rejection because the specification enables those of ordinary skill to practice the methods recited in claims 29 to 51 without undue experimentation.

Preliminarily, Applicants note that the Office Action indicates that "[t]he claims encompass treatment of neurodegeneration, stroke, Alzheimer's disease, amyotrophy, motor neuron damage, and acute CNS injury." (Office Action dated February 18, 2005, page 2). Applicants respectfully submit that this assertion is erroneous as the claims, in fact, recite methods of treating neurodegeneration, stroke, and Alzheimer's disease, and do not recite methods of treating amyotrophy, motor neuron damage, and acute CNS injury.

The enablement requirement is met if the specification enables those of ordinary skill in the art to make and use the subject matter defined by the claims without undue experimentation. *In re Angstadt*, 537 F.2d 498, 504 (C.C.P.A. 1976). The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. *Id.* Extensive experimentation is often necessary to practice inventions that involve unpredictable technologies, and such experimentation is not undue if the art typically engages in such experimentation. *PPG Indus., Inc. v. Guardian Indus. Corp.*, 75 F.3d 1558, 1564 (Fed. Cir. 1996).

The Examiner bears the burden of establishing a reasonable basis to question the enablement provided for the claimed invention. *In re Wright*, 999 F.2d 1557, 1562 (Fed. Cir. 1993). The Examiner must explain why he or she doubts the truth or accuracy of any

statement in a supporting disclosure, and must back up his or her assertions with acceptable evidence or reasoning. *In re Marzocchi*, 169 U.S.P.Q. 367, 360-70 (C.C.P.A. 1971).

Applicants respectfully submit that the specification enables those of ordinary skill in the art to practice the methods defined by claims 29 to 51, without undue experimentation, and the Office Action has failed to provide credible evidence or reasoning to the contrary. The specification teaches that hydroxamate compounds defined by the claims display pharmacological activities, including inhibition of cysteine and serine proteases, such as calpain. (See, for example, page 3, lines 12-13 and Example 16). The specification further teaches that calpain has been implicated in many diseases and disorders, including neurodegeneration, stroke, and Alzheimer's disease (see page 2, lines 4 to 6), and teaches that inhibition of calpain may be useful for the treatment of such diseases and disorders. (See page 6, lines 6 to 8).

The specification describes assays that can be used to evaluate the pharmacological activity and selectivity of the hydroxamates defined by the claims with respect to the inhibition of calpain. (See Example 16). The specification also teaches those of ordinary skill in the art how to formulate and administer the claimed hydroxamates for the treatment of the aforementioned diseases and disorders. (See page 10, line 22 to page 12, line 2).

Applicants respectfully submit that, in light of the direction provided in the specification, and the abundance of information that was available in the scientific and medical literature at the time the present application was filed, those of ordinary skill in the art would be able to make and use the full scope of the subject matter defined by claims 29 to 51 without undue experimentation.

In this connection, the state of the art at the time the application was filed indicates that calpain plays a role in, and thus may be implicated in the treatment of numerous diseases and disorders, such as neurodegeneration. For example, Bartus, R.T., *et al.*, *Stroke* 25:2265-70 (1994) (hereinafter "the Bartus Abstract")[1] indicates that calpain activity plays an important role in neurodegeneration associated with ischemic events and further indicates that calpain inhibitors provide a viable means of treating such neurodegenerative problems. Notably, the Abstract states the following:

[1] Attached hereto as Appendix A.

> These data provide the first evidence that a peripherally administered calpain inhibitor can protect against ischemic brain damage. They offer further support for an important role of calpain proteolysis in the brain degeneration associated with cerebral ischemic events and suggest that selective calpain inhibitors provide a rational, novel, and viable means of treating such neurodegenerative problems.

In addition, Li, P.A., *et al.*, *Neurosci Lett* 247:17-20 (1998)[2], indicates that calpain inhibitors protect against cortical neuronal damage. The art at the time of filing thus indicates that calpain inhibitors provide a viable means for treating neurodegeneration.

The state of the art at the time the application was filed also indicates that calpain inhibitors may be used to treat stroke. For example, Bartus, R.T., *et al.*, *J Cereb Blood Flow Metab* 14:537-44 (1994)[3] describes experiments demonstrating that calpain plays an important role in ischemia-induced neuropathology, and indicting that calpain inhibitors provide a powerful means of treating stroke and other ischemic brain incidents. Notably, the abstract states the following:

> Our data further support an important role of calpain in ischemia-induced neuropathology and suggest that calpain inhibitors may provide a unique and potentially powerful means of treating stroke and other ischemic brain incidents.

In addition, Markgraf, C.G., *et al.*, *Stroke* 29:152-8 (1998)[4] indicates that a calpain inhibitor protects against cerebral ischemia. The art at the time of filing, therefore, indicates that calpain inhibitors provide a powerful means of treating neuropathology associated with stroke.

Similarly, the art at the time of filing also indicates that calpain plays a role in Alzheimer's disease and calpain inhibitors may be used to treat Alzheimer's disease. For example, Iwamoto, N., *et al.*, *Brain Res* 561:177-80 (1991)[5] describes experiments conducted to investigate the possible involvement of calpain in the formation of plaques and tangles found in the brains of patients suffering from Alzheimer-type dementia (ATD), and states that "[t]hese data suggest that activation of calpain may be an important factor in the abnormal

[2] Attached hereto as Appendix B.
[3] Attached hereto as Appendix C.
[4] Attached hereto as Appendix D.
[5] Attached hereto as Appendix E.

proteolysis underlying the accumulation of plaques and tangles in ATD." In addition, Nilsson, E., *et al.*, *Neurobiol Aging* 11:425-31 (1990)[6] describes experiments that demonstrate a correlation between net calpain activity and the extent of neuropathological changes in the cortex of Alzheimer-degenerated human brain tissue. And Jordan. J., *et al.*, *J Neurochem* 68:1612-21 (1997)[7] describes experiments demonstrating that a calpain inhibitor prevented death of neurons induced by beta-amyloid, a protein whose presence in the brain is believed to induce or foster the formation of plaques characteristic of Alzheimer's disease. The art at the time of filing, therefore, indicates that calpain plays an important role in Alzheimer's disease, and also indicates that calpain inhibitors are effective to inhibit the death of neurons that occurs as a result of Alzheimer's disease.

The art at the time of filing thus indicates that calpain inhibitors may be used to treat neurodegenerative disease, stroke, and Alzheimer's disease. Accordingly, Applicants respectfully submit that the state of the art at the time the application was filed, coupled with the description provided in the specification, enable those of ordinary skill in the art to make and use the full scope of the subject matter defined by claims 29 to 51 without undue experimentation.

It is respectfully submitted that the Office Action has failed to provide credible evidence that those of ordinary skill in the art would have to engage in undue experimentation to practice the claimed methods. The Office Action asserts that the description provided in the specification, including *in vitro* data demonstrating the efficacy of compounds defined by the claims for inhibition of calapin I, fails to enable the claimed subject matter:

> [T]he reality is that, where protease inhibition is concerned, an attempt to extrapolate from such inhibition *in vitro* to therapy of one of the recited disorders is an exercise resulting in an "unpredictable" outcome.

(Office Action dated February 18, 2005, pages 2 to 3). The Office Action has presented the following arguments, and cited the following references, in an effort to support this proposition.

[6] Attached hereto as Appendix F.
[7] Attached hereto as Appendix G.

The Office Action asserts that the teachings of Chen, M., *et al.*, *Frontiers in Bioscience* 3:A66-75 (1998)(hereinafter "the Chen reference") raise considerable doubt as to whether calpain plays a critical role in the progression of Alzheimer's disease. (Office Action dated February 18, page 3). The Office Action concludes, based upon this interpretation of the teachings of the Chen reference, that any therapeutic benefit to be gained in the treatment of Alzheimer's disease through calpain inhibition is unpredictable. *Id.*

Contrary to the assertion made in the Office Action, Applicants respectfully submit that the Chen reference indicates that calpain plays a key role in the pathogensis of Alzheimer's disease, and *does not* raise any doubt that calpain plays a critical role in the progression of Alzheimer's disease. In fact, the reference states that "***calpain must play a key role in the pathogenesis of AD***, particularly in the processing of β-amyloid precursor protein." See Abstract (emphasis added). Although the Chen reference discusses disagreement in the literature regarding the particular role that calpain plays in the progression of Alzheimer's disease, the reference makes clear that it is undisputed that calpain is critical to the pathogenesis of the disease. Accordingly, the Chen reference does not support the inference made in the Office Action that treatment of Alzheimer's disease by calpain inhibition is highly unpredictable.

The Office Action further asserts that Kavita, U., *et al.*, *J. Biol Chem* 270:27758-65 (1995) (hereinafter "the Kavita reference") discloses that P338D1 macrophage lysate contains a factor that protects precursor IL-1 β from calpain proteolysis. The Office Action states that this teaching demonstrates that extrapolation from a cell-free incubation mixture to a simple *in vitro* system is unpredictable, and, accordingly, extrapolation from a cell-free incubation mixture to a physiological milieu must be unpredictable.

The Kavita reference, in fact, describes experiments aimed towards determining whether calpain plays a role in processing precursor IL-1β to mature IL-1β. The experiments described in the reference demonstrate that "calpain is not involved in the processing of precursor IL-1β *in vitro* or *in vivo*." (See Abstract). The Kavita reference does ***not*** describe inhibition of calpain activity, but, rather, describes experiments that attempt to determine whether IL-1β is processed by calpain. Accordingly, it is respectfully submitted that in contrast to the assertion made in the Office Action, the Kavita reference does ***not*** support the

proposition that data obtained with respect to inhibition of calpain in a cell-free system cannot be used to predict the outcome of calpain inhibition when cellular constituents are present in an *in vitro* system. The teachings of the Kavita reference, therefore, do not support the assertion made in the Office Action that inhibition of calpain activity *in vivo* is unpredictable based upon calpain inhibition *in vitro*.

The Office Action also cites Harriman, J.F., *et al.*, *J Pharmacology and Experimental Therapeutics* 294:1083-7 (2000) (hereinafter "the Harriman reference") for the proposition that certain compounds that inhibited purified calpain were ineffective in reducing calpain activity in renal proximal tubules (RPT), which might have been due to their limited uptake into RPT. The Harriman reference states, however, that "these results suggest that six of seven peptide calpain inhibitors are cell permeable" (Abstract) and suggests that "the inability of the calpain inhibitors to inhibit calpain activity may be due to the lack of specificity of the cellular calpain assay." (page 1087).

The calpain inhibition assay used in the experiments described in the Harriman reference measured the hydrolysis of SLLVY-AMC in RPT. The reference describes experiments conducted to determine whether the hydrolysis of SLLVY-AMC was due to several common proteases, rather than due to calpain activity. Although the reference indicates that hydrolysis of the substrate was not due to the proteases tested, the experiments do not rule out the possibility that some other entity was responsible for hydrolysis of the substrate, as the reference suggests. Accordingly, the failure of some of the compounds tested to inhibit calpain activity in RPT could have been due to the presence of a protease or other entity that hydrolyzed the substrate, and may not have been due to the failure of the test compounds to inhibit calpain activity. Accordingly, Applicants respectfully submit that the Harriman reference does not support the proposition that calpain inhibitory activity in intact cells is unpredictable.

The Office Action asserts that Saez-Torres, I., *et al.*, *Clin Exp Immunol* 121:151-56 (2000) (hereinafter "the Saez-Torres reference") discloses that peptide T inhibits T cell activation and cytokine production, but also discloses that peptide T is not effective in treating experimental autoimmune encephalomyelitis (EAE). The Office Action further asserts that Shields, *et al.*, *Proc Natl Acad Sci U.S.A.* 95:5768 (1998) (hereinafter "the Shields reference") indicates that calpain is upregulated and secreted by activated T cells, and cites

three additional references as evidencing a direct connection between calpain activity and activated T cells. The Office Action draws the inference, based upon these purported teachings, that therapeutic efficacy for treatment of inflammatory neurodegenerative disorders cannot be predicted on the basis of an *in vitro* assay.

The cited references, however, do not teach or suggest that peptide T is a calpain inhibitor. And they also do not teach or suggest that calpain inhibitors inhibit T cell activation and cytokine production. Accordingly, regardless of whether calpain is upregulated and secreted by activated T cells, experiments demonstrating the *in vivo* effectiveness or ineffectiveness of peptide T for the treatment of EAE are not relevant to determining the state of the art with respect to calpain inhibitors. It is respectfully submitted that the Saez-Torres reference, therefore, does not support the proposition that the treatment of inflammatory neurodegenerative disorders with calpain inhibitors is unpredictable.

The Office Action asserts that Steinberg, *et al.*, *The Scientist* 16:22 (2002) (hereinafter "the Steinberg reference") teaches that when mice that had developed Alzheimer's disease-like pathology were vaccinated, the plaques characteristic of Alzheimer's disease melted away, and favorable results were obtained in cognitive experiments with the mice. But when similar experiments were attempted in humans, the Alzheimer's disease symptoms worsened. The Office Action concludes that treatment of Alzheimer's disease is unpredictable, and states that "Applicants have shown no evidence that any of the claimed compounds can be used to treat Alzheimer's Disease." Applicants note that the Steinberg reference does not describe or suggest treating Alzheimer's disease with calpain inhibitors, and, accordingly, is not indicative of the state of the art with respect to the subject matter defined by the claims. Furthermore, as discussed earlier, art at the time of filing indicates that calpain plays a role in Alzheimer's disease, and further indicates that calpain inhibitors may be used to treat Alzheimer's disease. For example, as previously discussed, Jordan. J., *et al.*, *J Neurochem* 68:1612-21 (1997)[8] describes experiments demonstrating that a calpain inhibitor prevented the death of neurons induced by beta-amyloid, a protein whose presence in the brain is believed to induce or foster the formation of plaques characteristic of Alzheimer's disease.

[8] Attached hereto as Appendix G.

The art at the time of filing thus indicates that calpain inhibitors provide a viable means for the treatment of Alzheimer's disease.

The Office Action asserts that Haas, *et al.*, *J. Leukocyte Biology* 63:395-404 (1998) (hereinafter "the Haas reference") teaches that calpain inhibitors do not inhibit proinflammatory cytokine production, and concludes that the therapeutic efficacy of the claimed compounds for the treatment of inflammation is therefore unpredictable. The Office Action further asserts that Rossi, *et al.*, *J Biol Chem* 273:16446 (1998) (hereinafter "the Rossi reference") teaches that the propensity of compounds for inhibiting NF-κB cannot be predicted based upon their effectiveness for inhibiting calpain, and states that "a similar conclusion arises" from the teachings of the Rossi reference as can be drawn from the teachings of the Haas reference. The present claims do not recite methods for treating inflammation, however, and the Haas and Rossi references are thus not indicative of the state of the art with respect to the subject matter defined by the claims.

Accordingly, it is respectfully submitted that the Office Action has failed to provide credible evidence that reasons exist to doubt the objective truth of the teachings provided in the instant specification. The Office Action has therefore failed to establish that the present specification does not enable those of skill in the art to make and use the full scope of the subject matter defined by the present claims without undue experimentation. *In re Marzocchi*, 439 F.2d 220, 224 (C.C.P.A. 1971). Accordingly, Applicants respectfully request withdrawal of the rejection.

Alleged Indefiniteness

Claims 29 to 51 have been rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite for recitation of the word "about." Without conceding the correctness of the rejection, and to advance prosecution, claim 29 has been amended to remove each instance of the word "about." The rejection has been obviated, and Applicants respectfully request withdrawal thereof.

Conclusion

Applicants submit that the foregoing constitutes a complete and full response to the Office Action of record. Accordingly, an early and favorable action is respectfully requested.

Respectfully Submitted,

Date: May 20, 2005

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APPENDIX A

1: Stroke 1994 Nov;25(11):2265-70

Related Articles, Books, LinkOut

Calpain inhibitor AK295 protects neurons from focal brain ischemia. Effects of postocclusion intra-arterial administration.

Bartus RT, Hayward NJ, Elliott PJ, Sawyer SD, Baker KL, Dean RL, Akiyama A, Straub JA, Harbeson SL, Li Z, et al.

Alkermes Inc, Cambridge, MA 02139.

BACKGROUND AND PURPOSE: This research was performed to determine whether a selective inhibitor of the calcium-dependent protease, calpain, could reduce ischemia-associated brain damage when peripherally administered after a vascular occlusion. METHODS: A variation of the rat middle cerebral artery occlusion model was used. A range of doses of AK295 (a novel calpain inhibitor synthesized for this purpose) was continuously infused through the internal carotid artery, beginning 1.25 hours from the initiation of the occlusion. Rats were killed at 21 hours, and the infarct volume was quantified. RESULTS: Postocclusion (1.25-hour) infusion of the calpain inhibitor AK295 elicited a dose-dependent neuroprotective effect after focal ischemia. The highest dose tested (3 mg/kg per hour) afforded the maximum effect, illustrated by a 32% reduction in infarct volume 21 hours after the ischemia (vehicle, 81.7 +/- 4.7 mm3; AK295, 54.9 +/- 6.9 mm3; $P < .007$). CONCLUSIONS: These data provide the first evidence that a peripherally administered calpain inhibitor can protect against ischemic brain damage. They offer further support for an important role of calpain proteolysis in the brain degeneration associated with cerebral ischemic events and suggest that selective calpain inhibitors provide a rational, novel, and viable means of treating such neurodegenerative problems.

PMID: 7974554 [PubMed - indexed for MEDLINE]

APPENDIX B

☐ 1: Neurosci Lett 1998 May 8;247(1):17-20

Postischemic treatment with calpain inhibitor MDL 28170 ameliorates brain damage in a gerbil model of global ischemia.

Li PA, Howlett W, He QP, Miyashita H, Siddiqui M, Shuaib A.

Saskatchewan Stroke Research Centre, Faculty of Medicine, University of Saskatchewan, Saskatoon, Canada.

The newly-developed calpain inhibitor, MDL 28170 penetrates the blood-brain barrier and inhibits brain cysteine protease activity after systemic administration. This experiment was initiated to determine if the calpain inhibitor, MDL 28170 could, by these actions, reduce neuronal damage in an animal model of global cerebral ischemia in the gerbil. The calpain inhibitor, MDL 28170 (50 mg/kg), was initiated at 0.5 and 3 h of recirculation following 5min of global ischemia. Animals subjected to ischemia but without treatment or with vehicle treatment served as controls. Evaluation by light microscopy was carried out on paraffin-embedded brain sections of gerbils which were sacrificed 7 days post-operatively. The results show that the calpain inhibitor, MDL 28170, protects against cortical neuronal damage even if the treatment is delayed until 3 h after reperfusion. However, the neuroprotective effect of this agent is less pronounced in the hippocampal CA1 sector. The results suggest that calpain-mediated proteolysis plays an important role in neuronal death due to ischemia. However, additional mechanisms by which an increased intracellular calcium concentration leads to neuronal death may exist.

PMID: 9637399 [PubMed - indexed for MEDLINE]

APPENDIX C

1: J Cereb Blood Flow Metab 1994 Jul;14(4):537-44 Related Articles, Books, LinkOut

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Postischemic administration of AK275, a calpain inhibitor, provides substantial protection against focal ischemic brain damage.

Bartus RT, Baker KL, Heiser AD, Sawyer SD, Dean RL, Elliott PJ, Straub JA.

Alkermes, Inc., Cambridge, MA 02139.

Experiments were conducted to determine whether a potent, reversible calpain inhibitor could reduce the cortical ischemic brain damage associated with focal ischemia in the rat. AK275 (Z-Leu-Abu-CONH-CH2CH3), the active isomer of the diastereomeric mixture, CX275, was employed in conjunction with a novel method of perfusing drug directly onto the infarcted cortical surface. This protocol reduced or eliminated numerous, nonspecific pharmacokinetic, hemodynamic, and other potentially confounding variables that might complicate interpretation of any drug effect. Focal ischemia was induced using a variation of the middle cerebral artery occlusion method. These studies demonstrated a reliable and robust neuroprotective effect of AK275 over the concentration range of 10 to 200 microM (perfused supracortically at 4 microliters/h for 21 h). Moreover, a 75% reduction in infarct volume was observed when initiation of drug treatment was delayed for 3 h postocclusion. Our data further support an important role of calpain in ischemia-induced neuropathology and suggest that calpain inhibitors may provide a unique and potentially powerful means of treating stroke and other ischemic brain incidents.

PMID: 8014200 [PubMed - indexed for MEDLINE]

APPENDIX D

1: Stroke 1998 Jan;29(1):152-8

Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats.

Markgraf CG, Velayo NL, Johnson MP, McCarty DR, Medhi S, Koehl JR, Chmielewski PA, Linnik MD.

Hoechst Marion Roussel, Inc, Cincinnati, Ohio 45215-6300, USA.

BACKGROUND AND PURPOSE: Stroke patients often experience a significant temporal delay between the onset of ischemia and the time to initiation of therapy. Thus, there is a need for neuroprotectants with a long therapeutic window of opportunity. The efficacy of a potent, central nervous system-penetrating calpain inhibitor (MDL 28,170) was evaluated in a temporary model of focal cerebral ischemia to determine the window of opportunity for intracellular protease inhibition. METHODS: An ex vivo brain protease inhibition assay established pharmacodynamic dosing parameters for MDL 28,170. Middle cerebral artery (MCA) occlusion was accomplished by advancing a monofilament through the internal carotid artery to the origin of the MCA. Postmortem infarct volumes were determined by quantitative image analysis of triphenyltetrazolium-stained brain sections. RESULTS: Maximal inhibition of brain protease activity was observed 30 minutes after injection of MDL 28,170 with an estimated pharmacodynamic half-life of 2 hours. MDL 28,170 caused a dose-dependent reduction in infarct volume when administered 30 minutes after MCA occlusion. A window of opportunity study was conducted to determine the maximal delay between the onset of ischemia and the initiation of efficacious therapy. MDL 28,170 reduced infarct volume when therapy was delayed for 0.5, 3, 4, and 6 hours after the initiation of ischemia. The protective effect of MDL 28,170 was lost after an 8-hour delay. CONCLUSIONS: These data indicate that the therapeutic window of opportunity for calpain inhibition is at least 6 hours in a reversible focal cerebral ischemia model. This protection is observed despite the lethal hypoxic and excitotoxic challenge, suggesting that calpain activation may be an obligatory, downstream event in the ischemic cell death cascade.

PMID: 9445345 [PubMed - indexed for MEDLINE]

APPENDIX E

Localization of calpain immunoreactivity in senile plaques and in neurones undergoing neurofibrillary degeneration in Alzheimer's disease.

Iwamoto N, Thangnipon W, Crawford C, Emson PC.

MRC Group, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, U.K.

An antibody raised against the calcium activated neutral protease (calpain) was used to investigate the possible involvement of this enzyme in the formation of plaques and tangles in Alzheimer-type dementia (ATD) brain. Our results revealed the presence of a number of strongly stained calpain positive neurones in the normal human cerebral cortex and a loss of calpain positive cells in ATD brain. Furthermore, double staining experiments revealed that calpain immunoreactivity was present in cells undergoing tangle formation, and was also present in senile plaques. These data suggest that activation of calpain may be an important factor in the abnormal proteolysis underlying the accumulation of plaques and tangles in ATD.

PMID: 1797346 [PubMed - indexed for MEDLINE]

APPENDIX F

1: Neurobiol Aging 1990 Jul-Aug;11(4):425-31 Related Articles, Books, LinkOut

Calpain and calpastatin in normal and Alzheimer-degenerated human brain tissue.

Nilsson E, Alafuzoff I, Blennow K, Blomgren K, Hall CM, Janson I, Karlsson I, Wallin A, Gottfries CG, Karlsson JO.

Department of Psychiatry and Neurochemistry, St. Jorgens Hospital, University of Goteborg, Sweden.

The Ca2(+)-dependent neutral proteases calpain I and II as well as their specific inhibitor, calpastatin, were isolated from normal and Alzheimer-degenerated frozen human brain tissue. In the Alzheimer group calpain I activity was higher in cortex than in mesencephalon. The calpastatin activity was lower in cortex in both groups. This may implicate a higher Ca2(+)-dependent proteolysis in cortex compared to mesencephalon. In the Alzheimer group the cortical calpain II level decreased with an increasing degree of neuropathological changes. In the control group, the level of calpastatin decreased as the number of plaques and tangles increased. Evidence was obtained for a correlation of net calpain activity and the extent of neuropathological changes in cortex.

PMID: 2381502 [PubMed - indexed for MEDLINE]

APPENDIX G

1: J Neurochem 1997 Apr;68(4):1612-21

Related Articles, Books, LinkOut

Role of calpain- and interleukin-1 beta converting enzyme-like proteases in the beta-amyloid-induced death of rat hippocampal neurons in culture.

Jordan J, Galindo MF, Miller RJ.

Department of Pharmacological and Physiological Sciences, University of Chicago, Illinois 60637, USA.

We investigated the potential role of different proteases in the death of cultured rat hippocampal pyramidal neurons induced by beta-amyloid (A beta) (25-35). Both A beta(25-35)- and staurosporine-induced death of these neurons appeared to involve apoptosis, as indicated using Hoechst 33342 and terminal dUDP nick end labeling staining, whereas NMDA-induced death appeared more complex. Two irreversible inhibitors of the interleukin-1 beta converting enzyme (ICE) and related proteases, Z-Val-Ala-Asp-CH2F and acetyl-Tyr-Val-Ala-Asp-chloromethyl ketone, blocked neuronal death produced by A beta(25-35), staurosporine, and NMDA to differing extents. Furthermore, MDL 28,170, a selective inhibitor of the calcium-regulated protease calpain, also inhibited death induced by all agents. A beta (25-35) and staurosporine stimulated the breakdown of the protein spectrin, a calpain substrate. Spectrin breakdown was inhibited by MDL 28,170 but not by ICE inhibitors. Leupeptin was only effective in preventing NMDA-induced death. These results support the role of apoptosis in neuronal death due to A beta(25-35) treatment and also suggest a role for calcium-regulated proteases in this process.

PMID: 9084433 [PubMed - indexed for MEDLINE]